PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C05F 1/00, 3/00, 9/00, 11/00 — A1

(11) International Publication Number: **WO 96/28400**

(43) International Publication Date: 19 September 1996 (19.09.96)

(21) International Application Number: PCT/US96/03775

(22) International Filing Date: 13 March 1996 (13.03.96)

(30) Priority Data:
08/402,711 13 March 1995 (13.03.95) US

(71)(72) Applicant and Inventor: FINNEY, Jerry, W. [US/US]; 801 West Broadway, Bloomfield, NM 87413 (US).

(74) Agent: BECKER, Robert, W.; Robert W. Becker & Associates, Suite 200, 5300 Sequoia N.W., Albuquerque, NM 87120 (US).

(81) Designated States: AT, AZ, CA, CN, DE, EE, GB, JP, KZ, LT, LV, MX, RU, SE, TM, UA, UZ, VN, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: BIOMASS REDUCTION AND BIOREMEDIATION PROCESSES AND PRODUCTS

(57) Abstract

An aerobic, thermophilic, alkalinic composting process for biodegrading waste products and producing byproducts which are useful for agriculture fertilizer as well as for bioremediating organic and hydrocarbon products. The composting process has multiple steps including an early step of creating extremely high temperature and extremely high pH to stress bacteria to enable them to induce enzymatically catalyzed hydrolysis of waste products or organics or hydrocarbons in the presence of water. This is accomplished at the end of a first composting stage, employing indiginous bacteria under ambient conditions, by adding alkaline materials to the compost pile to reach a pH of at least 10, followed by a second composting stage, and then adding nutrients to the compost pile while maintaining a high level of moisture in the compost pile during the final composting stage. Composted products are unique and are suitable for use in subsequent processes for bioremediating organic and hydrocarbon contaminant materials *in situ* or *ex situ*. *In situ* subsurface bioremediation of soils is benefited by combining DC electrical currents to help propagate treating materials through contaminated soils or clay.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

BIOMASS REDUCTION AND BIOREMEDIATION PROCESSES AND PRODUCTS

Technical Field

This invention relates to the field of biomass remediation or composting processes for the production of a useful microbial product for either subsequent bioremediation processing of various substrates or direct application to soil as an agricultural fertilizer. The process involves a thermophilic alkalinophilic reaction, involving predominantly aerobic microbes, which stresses the microbial population in the presence of high moisture and high pH conditions during an initial reaction step and wherein subsequent reaction at high moisture, lower pH and added nutrients enables the surviving microbial population to perform a composting operation in a novel operation wherein the aerobes break down or digest the waste substrate materials without conventional stirring, turning or agitation of the compost pile to aerate it and without the need for otherwise directly introducing air or oxygen by conventional mechanical devices such as pumps or blowers. This invention also relates to the field of bioremediation of contaminated soils or other industrial wastes and the production of valuable fatty acids and amino acids.

Background of the Invention

The existence of environmental contamination is recognized as one of the most serious problems of governmental agencies as well as private land or property owners and users. A major problem of all communities is the disposal in a safe sanitary manner of usual municipal wastes. Even after recycling screening to sort out recyclable glass, metal, paper and plastic products, there remain vast amounts of mixed hazardous and benign materials destined for land fills, sewage disposal plants or the like,

which have to be treated for a variety of reasons, such as elimination of hazardous materials and pathogens and any materials which present a risk of contamination by seepage or escape into exposed water bodies, soils or ground waters and subsequent undesired displacement or migration into other uncontaminated and often inaccessible areas. Bioremediation of such contaminates and waste products is now being pursued as one likely way of solving contamination problems. However, prior to the present invention, the processes and products being used never reached the ideal parameters for safe, low cost, volume reducing and easily achieved results attainable with indigenous bacteria operating to bioremediate usual municipal waste products. Environmental contamination at a waste or other site presents many problems even when the contamination is accessible such as at surface dumping or spills of contaminants on soil, but inaccessibility due to deep underground leaching or seepage of contaminants or penetration of such contaminants into soils beneath buildings, pavements, runways or impenetrable rock or soil formations compounds the problems in any attempts at remediation. Such contamination by seepage is not at all limited to municipal waste disposal, and often occurs due to undesirable release or escape of hazardous materials from refineries, chemical manufacturing plants, manufacturing plants using, or having as waste by-products, various chemicals and paints, and is often due to spills occurring during transportation of hazardous materials. Although federal, state and municipal laws and ordinances make attempts to control environmental contamination, there are thousands of sites where contamination has occurred by accident or mistake, or often far in the past before the recognition of environmental problems as they exist today, where site

remediation is both publicly and privately needed.

One of the seemingly simplest methods of treatment of municipal waste is the conventional windrow piling and composting of waste where indigenous bacteria react with and chemically decompose the waste. Such processes are well known were aerobic thermophilic reactions are effected during periodic turning or stirring of the pile to aerate it to provide oxygen the support the aerobic process. Other known methods for aeration include maintaining porosity in the pile by introduction of wood chips and forced introduction into the pile of air or oxygen through perforated pipes or supporting grates. The attendant mechanical equipment and the operators therefor and the maintenance thereof is a substantial cost factor in such composting operations. If landfill capacity for the residual composted products is limited or non-existent, disposal of such products is a major problem.

Another residual disposal problem occurs in connection with sewage sludges which creates psychological as well as physical disposal problems in connection with its use and/or disposal under safe healthful conditions.

To further elaborate on the background of the present invention, the following cited articles are cited and incorporated by reference in this specification:

Abelson, Reed, Bugs clean up their act,
p. 144, Forbes, Sept. 28, 1992

Bouwer, E.J. Bioremediation of Organic contaminants
in the Subsurface, Chapter 11,
pp. 287-318 of environmental Microbiology
Edited by Ralph Mitchell, Wiley-Liss 1992

Feinstein, Melvin S., Composting in the Context of
Municipal Solid Waste Management, Chapter 14,

pp. 355-374 of Environmental Microbiology
Edited by Ralph Mitchell, Wiley-Liss 1992

Gillis, Anna Maria, Shrinking the trash heap
p. 90 (4 pp.), BioScience, V. 42, N. 2, Feb., 1992

Glass, David J., Waste Management
p. 5 (4 pp.), Environment, V. 33, N. 9, Nov., 1991

Madsen, E. L., Sinclair, J. L., Ghiorse, Wm.C.
In Situ Biodegradation: Microbiological Patterns
in a Contaminated Aquifer

Stover, Dawn, TOXIC AVENGERS
p. 70, (6 pp.) Popular Science, July, 1992

Advantages of composting by using municipal sludge mixed with waste are pointed out by the enclosed article of Gillis. This article points out that although great improvement in the growth of various plants and vegetables is indicated, it may be necessary to evaluate the use of most compost products on the basis of crop type, irrigation and geographical regions to establish the optimum rate at which the composting material should be applied to the top soil. It is anticipated that improved processes like those of the present invention may overcome some of the existing handicaps in safely getting rid of hazardous waste which may be banned by regulation of EPA or other federal state or local laws or regulations which may restrict the disposal of hazardous waste at landfills or composting sites.

Although it has been recognized that indigenous organisms can produce bioremediation of, for example, municipal waste sites where composting takes place under aerobic conditions, the present invention uses indigenous organisms, but the inventive processing steps achieve an enzymatically catalyzed reaction, which not only reduces the amount of turning to aerate

the compost piles, or the expensive process of pumping air or oxygen into the pile, but also provides a very fast reaction process where the composting operation can be essentially completed in little more than a month which is typically unheard of in popular composting processes.

Disclosure of the Invention

The present invention both from a processing standpoint as well as in the use of derivatives from the basic composting operation is useful in solving many of the problems identified in the above articles, including but not limited to the breakdown and disposal of municipal solid waste, bioremediation of hazardous materials, safe breakdown and disposal of waste products at manufacturing sites, in situ bioremediation or surface contaminated soil, in situ bioremediation of subsurface soils and liquid bodies, breakdown of long chain carbon compounds into shorter carbon chain compounds, the derivation of useful amino and fatty acids at low cost as bioremediation byproducts and the generation of a pathogen free fertilizer as a composting byproduct with an improved plant nutrient retention time accompanied by improved water retention when applied to soil for agricultural purposes. If the size of the bioremediation project is sufficiently small, ex situ remediation in barrel-like containers is convenient and practical, particularly for conversion of substrate materials to more useful products in a manufacturing environment.

Another important aspect of this invention relates to improved methods for remediation of subsurface soil contaminants. Microbe products produced in accordance with the invention are used for in situ bioremediation, making use of DC electrical currents to force bacteria through soils which may be clay or other soils normally difficult to penetrate. Nutrients are

added while applying the electrical charges.

Brief Description of the Drawings

The features of the invention, and its technical advantages, can be seen from the following description of the preferred embodiments together with the claims and the accompanying drawings, in which:

FIGS. 1-4 are charts showing test results of the action of bacterial strains of the present invention on trichlorothylene (TCE);

FIG. 5 illustrates two electrically interconnected subterranean wells connected to a DC power source and to a bacterial and nutrient supply tank;

FIG. 6 is an expanded vertical section of the negatively charged well of FIG. 5;

FIG. 7a is a plan diagram of multiple locations for electrically interconnected subterranean wells as in FIG. 5, arranged at the center of and in a circle for subsurface bioremediation;

FIG. 7b is a plan diagram similar to FIG. 7a with the well locations at opposite sides of a rectangle;

FIG. 8 is a vertical section through a tank used for bioremediating the hazardous waste toxaphene;

FIG. 9 is a diagrammatic section of a pit containing hydrocarbon contaminated soil and with a central tube for extracting bioremediation products;

FIG. 10 is a diagrammatic section of a bioreactor for disposing of manufacturing wastes or for manufacturing useful products by bioconversion.

Description of Preferred Embodiments

While not being bound by theory set forth herein as

to why the processes and products detailed herein perform in unexpected ways and achieve unexpected results, it is believed that the initial step of stressing the bacteria population at high temperature and at high pH in the presence of high moisture in a compost pile results in, at the end of approximately two days, a substantially pathogenic-free population of bacteria having the capability of continuing to repopulate the pile and break down the waste products therein during a one to two month period after the initial stressing step. During this breakdown period nutrients and water are added to support an enzymatically catalyzed reaction at the interface of the bacteria, the water, the nutrient and the waste substrate in such a way that oxygen needed for what is deemed an aerobic process is derived from the water being continually added to the pile. To the extent that elemental or molecular oxygen is not being supplied throughout the composting process by aerating or pumping oxygen into the pile, the aerobes of the pile are reacting in an anaerobic environment. The required oxygen under the present circumstances is not primarily supplied in gaseous form, but is made sufficiently available at the reaction interface, where hydrolysis of waste takes place in the presence of water, for the aerobic-type reaction to take place. Water is also regenerated in the pile by the breakdown of organic compounds from which carbon combines with oxygen to form carbon dioxide and hydrogen combines with oxygen to form water. A deleterious hydrocarbon may be broken down as part of a composting operation using the present process or as a substrate specific material being acted upon by a similar process using bacteria derived from the composting process. The use of composting derived bacteria is preferable to provide a high degree of metabolic activity supported by the enzymatically catalyzed

generation of oxygen to accelerate any bioremediation action. The process for producing these composting-derived bacteria can be repeated at will using readily available waste, sewer sludge, horse manure, water and indigenous bacteria combined as set forth elsewhere in this specification.

Although the genus bacillus is one of the most studied microorganism genera, this invention has demonstrated the unique ability of strains of this genus of bacteria, found as end products of this invention, to not only be extremely prolific and rapidly populate (see FIG. 8), but also to have properties of their enzymes for catalyzing a reaction involving water, nutrients and contaminated substrates to provide biomass degradation and bioremediation that is able to convert typical municipal wastes into benign and useful ingredients such as chemical byproducts or agricultural fertilizer. In addition, the resulting product is also capable of enzymatically catalyzing the bioremediation of chlorinated hydrocarbons and long chain hydrocarbons, particularly petroleum hydrocarbons. The process involved in this invention provides in-situ bioremediation in a manner not believed to have been achieved in the prior art. The processes have the great economic benefit of not requiring typical turning or rototilling of huge windrows of waste products as typically occurs in composting operations. The invention also enables bioremediation of subterranean and inaccessible contaminated soil without requiring excavation or removal of the soil to gain access to the contaminants as has been done in some instances in operations where external reactor vessels are used to convert toxic chemical compounds in the presence of microorganisms that react therewith.

This invention provides a low cost method of

producing large quantities of enzymes which are instrumental in enzymatically catalyzing the bioremediation of waste and hydrocarbon materials without the need for introduction by mechanical equipment of quantities of air or oxygen typically used to support aerobic bioremediation reactions.

A unique property of the microbes and enzymes of the invention is that bioremediation of contaminated materials appears to be taking place using what are usually considered to be aerobic bacteria, but the aerobic bacteria are operating in what is normally considered to be an anaerobic environment. That is, there is no supply of air or oxygen to the reaction activity, but rather the nutrients, contaminants, and water react with the microbes, and enzymatic catalyzation of a reaction effectively bioremediates the contaminants. If a nutrient does happen to contain oxygen, i.e. NO_3 nitrates, it should by pointed out that the present process is not intended to be dependent on any availability of oxygen from such a nutrient.

The percentages of strain types of aerobes and anaerobes in a composting product of the invention are shown in Table 1, below:

Strain	Compost
Aerobes	
1	10%
2	50%
3	10%
4	10%
5	20%
Anaerobes	
6	40%
7	40%
8	20%

Table 1: Percentage of Strain Types in Compost Sample

Evidence of the ability of compost-generated aerobes of the present invention to perform a bioremediation action in an anaerobic environment appears in the laboratory data of Table 2, below, and FIGS. 1-4 showing that the aerobic strains grow better on Trichlorethylene under anaerobic conditions and are actually inhibited by the TCE under aerobic conditions.

Strain	Aerobic 24 Hours	Aerobic 48 hours	Anaerobic 48 hours
1	Inhibited	Inhibited	Fair
2	Inhibited	Inhibited	Inhibited
3	Inhibited	Inhibited	good
4	Inhibited	Inhibited	good
5	Inhibited	Inhibited	fair
6	Fair	Inhibited	inhibited
7	Inhibited	Inhibited	fair
8	Inhibited	Inhibited	minimal

Table 2: Growth of Aerobic Strains in the presence of TCE under Aerobic and Anaerobic Conditions

The present invention is able to produce a bioremediating product from the sites of conventional municipal waste disposal locations and the product material, which is useful either as a fertilizer or for ensuring bioremediation of other materials or products, has a long useful shelf life. Tests performed in using this product derived from composting operations have indicated a useful shelf life is well over one year.

Composting Process

In the preferred embodiment of the invention which is initially used for composting municipal waste, the initial biomass comprises trash, horse manure and secondary sewage sludge mixed together. The sludge is preferable free of oily dirt and has about 18 percent solids. Quicklime or flyash is added to raise the pH to 12 or above and to achieve a rapid autocatalytic heat rise in a few seconds to about 200 degrees F. Water is added to keep

the solids in the pile at about 18 percent. This maintains the moisture content at about 82 percent which is sufficient saturation to make the aerobic bacteria react in what is essentially an anaerobic environment. The high thermal and alkaline conditions help to stress the bacteria so that after 24 to 36 hours the bacteria are set in their ways, i.e. the population of bacteria assumes a stable character, and are ready for the further addition to the composting pile of nutrients which cool the pile and prepare it for a one to two month period to complete the biomass reduction and biodegradation of all the organic materials in the pile.

After the first addition of nutrients the pile can be left standing for 7 to 10 days with only clear water being added as required to keep the moisture level high. After this time the pile is turned once to get the outside to the inside. Then more nutrients are added and the pile can stand idle for two more weeks. Then the pile is turned once to get the outside to the inside. Then more nutrients are added and the pile can stand idle for two more weeks. Then the pile is turned a third time and further nutrients added. Then the pile continues the enzymatically catalyzed reactions until the completion is signalled by a gradual temperature drop to about 80 to 90 degrees F. at the end of the degradation process. This temperature is one indicator of the completion of the process. Another such indicator is the presence of a fine granular or silt-like consistency of the resulting organic soil.

It should be noted also that the high temperatures and high pH during the first two days of stressing the bacteria effectively kills the pathogens in the pile.

The composting process of the preferred embodiment is performed generally according to the following

example:

EXAMPLE 1

A compost pile mixture of trash, horse manure, secondary sewer sludge, cellulose, carbohydrates, and other typical municipal organic waste is arranged in a critical mass, i.e. 75 cu. yds., with an initial pH of 7 or 8 so as to start an autocatalytic exothermic reaction due to microbial activity in the presence of moisture. Such a compost pile typically contains indigenous microorganisms which participate in an aerobic, thermophilic and alkalinophilic reaction which is a fundamental aspect of the present process.

The mixture has sufficient organic and inorganic nutrients to support the temperature- raising reaction including inorganic compounds of nitrogen, phosphorus, sulfur and potassium. Upon reaching a temperature of 115-140 degrees F. after about 10 hours, the activity of thermophilic alkalinophilic microorganisms is increased or accelerated by the additional mixing of basic or alkalinic material such as KO, CaO (lime), NaO, MgO or flyash in sufficient quantity to increase the pH level of the initial mixture to about 12 or higher. It is desirable to wait for the temperature rise before adding the alkaline material because there is a danger of killing off desirable microbes otherwise.

After adding the pH-raising alkaline material, the exothermic reaction continues with a substantial increase in temperature. In addition to the aerobic organisms that require molecular oxygen, facultative organisms are forced to go aerobic. The reactions maintain dissolved oxygen sufficient (i. e. at least 1 ppm.) to enable survival of aerobic organisms. The combined high pH and high temperature is believed to kill pathogenic bacteria whereas primarily aerobic thermophilic strains of

microbes, including aerobes of the genus Bacillus, and their high energy enzymes continue to be reproduced or generated. These surviving microorganisms and enzymes are believed to go through an evolutionary process which keeps the microbes alive and makes the final composition of living bacteria and enzymes, and co-produced amino and fatty acids, useful products.

The primary enzymes produced during the degradation process are lipase and protease, but lignase is also produced. The exact nature of mutation or recombination which is believed to take place has not been identified.

The temperature rise of the new mixture after pH alteration may be as high as boiling water. However the useful surviving microorganisms were found to have survived even substantially higher temperatures in the presence of some moisture.

In addition to surviving at high temperatures, the surviving microorganisms were also found to survive at temperatures near the freezing temperature of water.

The process is continued for a sufficient time, approximately 24 hrs., for the compost pile to reach a desired recycled non-contaminated condition. Upon the addition of the nutrients, ammonium sulfate 21-0-0, phosphate 0-45-0, potassium sulfate 0-0-60 in equal amounts by weight, the temperature drops and the pH drops to a near-neutral level of 7-7.5. The microorganisms can continue to multiply in the presence of moisture and nutrients and the end product includes high energy enzymes with improved cleavage ability for bioremediation of hydrocarbon contaminants from soil and the resulting production of innocuous water and CO_2.

Throughout this process the combined waste

materials and additives are solids which facilitates handling the materials.

Metal ions in the reacting mixture do not impede the formation of useful enzymes which are retrievable as useful products for bioremediation of hydrocarbons and for regenerating biofouled activated carbon used in other processes.

In a similar alternative embodiment the trash and horse manure can be added to the composting mixture after the stressing period. Preferable the horse manure is added first so that the bacteria therein are subjected to the high temperature and high pH before the addition of trash which has a high paper content tending to bring the pH down rapidly.

In both of these embodiments water is continually sprayed on the piles to maintain the high moisture. Similarly in both embodiments the nutrients are added at about 48 hours after the beginning of the stress period with high pile moisture content until composting is complete.

Typically, here, as well as in other composting described herein, there is a gradual temperature drop to about 80 to 90 degrees F. at the end of the degradation process. This temperature is one indicator of the completion of the process. Another such indicator is the presence of fine granular or silt-like consistency of the resulting organic soil.

Although the molecular structure of the microbes and their enzymes resulting from the processing steps of the present invention are not specifically known, it is recognized that these microbes and their enzymes have been subject to extremes of temperature and pH during the aerobic metabolic process taking place. For at least a short time of the order of 24 hours the microbial products are very highly stressed and it is believed that

only the highly thermophilic microbes survive. It is believed that not only are pathogenic microbes killed or permanently denatured by the heating, but also it is believed that even those of the thermophilic microbes which may be denatured at temperatures of the order of 100 degrees C. may be renatured during the cooling achieved after about 48 hours upon the addition of the nutrients. After nutrient addition not only does the temperature drop but also the pH also drops from somewhere in the vicinity of 12-14 pH to around 7-8 pH.

Compost Product Analysis

The results of a laboratory total plate count analysis to identify the types of bacteria in the compost produced material resulted in identifying the strains listed in Table 3 below:

Strain Name	Primary Identification GCFAME	Sim. Coef.	Dist. Coef.	Primary Identification Biolog (TM)	Plate Type	Sim. Coef.	Dist. Coef.
3238-1	*Bacillus circulans CLIN*	.489	3.864	closest species *Bacillus insolitus*	GP	.921	.962
3238-2	*Bacillus coagulans*	.143	6.376	*Bacillu pasteurii*	GP	.791	2.976
3238-3	*Bacillus latersporous*	.530	3.642	*Bacillus insolitus*	GP	.900	1.260
3238-4	*Staphylococcus aureus*	.607	3.232	*Bacillus pasteurii*	GP	.440	6.126
3238-5	*Micrococcus varians*	.350	4.684	closest species *Bacillus pasteurii*	GP	.169	11.916
AN 3238-6	*Prevotella veroralis*	.001	10.900				
AN 3238-7	*Rothia denticariosa*	.016	8.516				
AN 3238-8	*Staphylococcus epidermidis*	.005	9.753				

Table 3: Strain Identification of Aerobes and Anaerobes in a Composting Product

These results were obtained using standard bacterial

plate count processing to isolate the strains. Five aerobe strains and three anaerobe strains were found in a sample of compost product. Following isolation, the strains were individually placed onto TSA. The TSA plates were processed after 24 hour incubation by [Method 1 - Standard GC-FAME]) and Clinical Aerobe (CLIN[rev.3.80]) or the anaerobic [ANER1]GC-FAME databases. Subsequently the aerobic strains were prepared for Bilog (TM) analysis by suspending them in sterile saline and loading the solutions into the appropriate microtiter plates (Gram negative or Gram positive). The plates were incubated for 24 hours and then examined against version 3.5 of the Biolog (TM) database using an automated microplate reader. The Similarity and Distance Coefficient for each strain shown in Table 3 refers to the similarity and distance to the hypothetical "mean" organism in the database. The database organism has a similarity coefficient of one and a distance of zero. The closer a strain is to respective coefficients of one and zero, the more closely it matches the means organism of the database. A good match is one with a similarity coefficient greater than 0.5 and a distance coefficient of less than 7.

Table 4, below shows the growth rate of aerobic and anaerobic bacteria of the invention:

	24 Hours	48 Hours	Types
Aerobic compost	5.06×10^5	8 .15 x 109	5
Anaerobic compost	7.20×10^4	1.18×10^5	3

Table 4: Total Heterotrophic Plate Count

Bioremediation and Fertilizers

A secondary aspect of the present process is to mix a concentrated portion of the solid material produced by the above process and containing surviving living or active organisms with water and use the liquid mixture to inoculate and bioremediate soil contaminated with hydrocarbons (as from fuel spills or tank leakage) and chlorinated hydrocarbons (pesticides, PCBs, etc.).

Fertilizers:

These processes of composting and bioremediation combine about 50 percent microbial metabolic processes and about 50 percent synthetic or enzymatic processes, particularly extracellular processes. When the process of the preferred embodiment is used to produce a byproduct containing gypsum for agricultural fertilizer application, the nutrients used are 21-0-0 ammonium sulfate, 0-45-0 super triple phosphate and 0-0-60 potassium in even volumes. These additions drive the pH down to neutral and start the buildup of gypsum ($CaSO_4$-$2H_2O$) as the reaction continues. Gypsum is a valuable fertilizer for which the retention period is desirably longer than most fertilizers and which also helps to retain moisture in the soil. Depending on the gypsum requirements for particular crop and soil types, the percentage of gypsum in the final composted product can be varied over a range of about 3 to 12 percent by varying the quantity of nutrients added. Phosphogypsum will also be present in a significant amount and its relative degree of presence can be varied by changes in the relative amount of (0-45-0) super triple phosphate which is used.

Bioremediation:

As pointed out above, the composting end product

from the preferred composting process may, in lieu of use as an organic fertilizer, be used for bioremediation of various organic and hydrocarbon substrate materials. The generation and recovery of useful products such as amino and fatty acids provides a significant economic benefit derived from these processes.

An additional important feature of the present invention is that the bacteria achieved by the initial composting operation are not selective, i.e., they can be made to bioremediate a large variety of different hazardous or contaminating materials and thus provide a more inclusive process of generic application without the necessity of extensive analytical preparation to determine the location of contaminants, the type of bacteria needed to bioremediate the contaminants and an expectation that most contaminants can be treated by the products of this invention to break them down to benign or innocuous materials such as water and carbon dioxide and potentially beneficial acids.

If the end composted product of the preferred embodiment is to be used as a bioremediating agent for subsequent bioremediation of organic or hydrocarbon materials, the nutrients added for such a subsequent process are preferably (46-0-0) urea, (32-0-0) ammonium nitrate, (0-45-0) phosphate and (0-0-30) potash with the nitrogens being 10 to 1 by weight relative to the total phosphorus and potassium. Such a subsequent process may be of the type shown and described in connection with FIGS. 5-7 and 9-10. For in-situ bioremediation in soils the moisture content can drop as low as about 20 percent by weight and still preserve the necessary conditions for an enzymatically catalyzed bioreaction.

In-Situ Bioremedeation of Paraffin Crude Oil/Water Mixture in Open Pit

Another embodiment of the invention involves the bioremediation of paraffin crude oil in a mixture of other composting materials. This paraffin crude is typically in an earthen pit and is mixed with water. As a first step, secondary digester sludge having about 3 to 5 percent moisture by weight is mixed with horse manure in a pile using the sludge as the wetting agent. After mixing, the overall moisture in the pile reduces to about 50 percent by weight. Then whole trash, mainly paper products such as magazines, cardboard boxes, paperback books and newspapers are added to the pit to absorb the crude oil and water forming an oily trash mix with a resulting average hydrocarbon content of 30 percent (300,000 ppm.) Total Petroleum Hydrocarbons (TPH) per the 418.1 Infrared Spectrometry Test. Then both mixtures are mixed together to form a mixture having a "jello" like consistency. After about ten hours of bioreaction the overall mix rises in temperature to about 130 degrees F. After 48 hours the temperature reaches about 160 degrees F.

The amounts of the mix ingredients are 110 yds. (cu. yds.) horse manure, 2000 gals. sludge, 50 yds. trash and 25 yds. of the paraffin crude oil.

After 10 days the oily consistency disappears and the TPH drops to about 5000 ppm. The pile may be turned at that time mainly to get outside layers to the inside of the pile. Under the same type of high moisture content of at least 60 to 75 percent the overall mixture is biodegraded with the hydrocarbons being bioremediated by a continuing enzymatically catalyzed microbial action. After 30 days the pile temperature levels off at

about 130 degrees F. and the pile is turned once more, again just to get the outside to the inside for better action. At this time the TPH is down to 2000 to 3000 ppm. After 60 to 90 days the TPH levels dropped to less than 100 ppm. which is acceptable as a level of satisfactory bioremediation of the hydrocarbons.

In-Situ Bioremediation of Subsurface Soil

Another embodiment of the invention relates to the use of an aerobic microbial product for bioremediation of subsurface or underground contaminants such as spills or seepage in the vicinity of buried storage tanks, as typically used for storage of gasoline or other liquid products which are deemed hazardous contaminants due to their ability to migrate through soil or other ground materials and mix with and contaminate ground water to make the latter non-potable, or at least less useful. There are many such sites throughout the United States which have been identified and for which there is a need for bioremediation before the contaminating liquid has migrated too far. Many of such sites are already provided with multiple test wells of small diameter in a predetermined pattern to enable test samples to be drawn from each well to repeatedly monitor and indicate the extent of or change of the contamination. The contaminated areas of such sites are often not readily accessible due to being at substantial depths, perhaps hundreds of feet underground, or because they are under pavements, buildings or other structures which for various reasons must not be disturbed.

Although energized electrodes have been previously used in such sampling well holes, the present invention contemplates assuring bioremediation at such sites is successfully and expediently performed by assuring the presence of suitable

microbes and enzymes with needed nutrients and enough water moisture to enable aerobic reaction of the microbial product with nutrients and the contaminating substrate which is to be converted to benign products.

As seen in FIGS. 5-7 this embodiment of the invention contemplates the use of at least two spaced vertically extending wells or bore holes of small diameter extending to a depth at least equal to the depth of the deepest layer portion of any contaminated substrate layer.

Referring to FIG. 5, subsurface bioremediation system 10 is installed in earth 12 having surface 14 and contaminated area 16 located at a depth of from about five feet to about ten feet below surface 14. Positively charged well 18 is vertically disposed, extending from surface 14 downwardly through contaminated area 16, in the instant case for a total of about ten feet. Well liner 20 extends the length of well 18, fitting against the inside surface of the borehole thereof, well liner 18 having at its lower end an endcap 22. Well liner 20 has perforations 24 located along its lower portion within the area of contaminated area 16, i.e., the lower five feet thereof. Well liner 20 may be constructed from polyvinyl chloride (PVC) pipe. Perforations 24 are of about 1/4 inch in diameter and extend through the wall of well liner 20. The perforated portion of the PVC pipe may be conventional well monitoring PVC pipe having multiple small arcuate circumferentially extending slits along the length of the perforated pipe portion. Positively charged coaxial electrode 26 is located within well 18, concentric with well liner 20 and extending substantially the entire length thereof, the lowermost five feet of electrode 26 being in communication with contaminated earth 16 by means of perforations 24 in well liner 20. Coaxial electrode 26

is formed of from 3//4 to 1 inch diameter standard copper tubing and is perforated with apertures 28 of approximately 1/8 in. diameter at 3 inch intervals over the portion of the length of electrode 26 corresponding to the perforated portion of well liner 20-, preferably for a minimum of 2 feet of the perforated portion of liner 20. Electrode 26 is positively charged through its connection with DC electric generator 30 by means of wire 32.

Negatively charged well 34 is spaced from positively charged well 18 and is preferably located at a location extending into a portion of contaminated area 16 remote from positively charged well 18. Negatively charged well 34 may be identical in configuration to positively charged well 18. Well 34 comprises well liner 36 having end cap 38, the lower five feet thereof having perforations 40 extending therealong. Negatively charged concentric electrode 42 may be identical in configuration to positively charged electrode 26, and is located within well 34, extending substantially the entire length thereof, the lowermost five feet of electrode 42 being in communication with contaminated earth 16 by means of perforations 40 in well liner 36. Electrode 42 is constructed of standard copper tubing in a similar manner to electrode 26 and is perforated with apertures 44 similarly disposed as apertures 28 in electrode 26. Electrode 42 is negatively charged by means of its connection with DC generator 30 by means of wire 46. The upper portion of negatively charged electrode 42 is connected with tank 48 by means of hose 50 for fluid flow between tank 48 and electrode 42.

FIG. 6 is an expanded view of negatively charged well 34, negatively charged electrode 42, and its connection with hose 50.

FIG. 7a is a plan view of a typical layout of wells as

in FIG. 5 for a circularly disposed contamination area 16, wherein negatively charged well 34 is located in the center of the contamination area 16 and a plurality of equally spaced positively charged electrodes are located near the perimeter of the contamination area 16 and equidistant from centrally located negatively charged well 18. Although the relative charges may be reversed, it is most convenient to provide liquid from tank 48 (See FIG. 5) to a single negative electrode. In the illustrated case, six well pairs are formed by six positively charged wells 18 and a single negatively charged well 34.

FIG. 7b is a plan view of a typical layout of wells as in FIG. 5 for a rectangularly disposed contamination area 16, wherein a plurality of negatively charged well 34 are located along one side of contaminated area 16 and a corresponding number of positively charged wells 18 are located along the opposite side of contamination area 16. In the illustrated case, three well pairs are formed by three positively charged wells 18 and three negatively charged wells 34.

In operation, nutrients, microbes and water to support subsurface bioremediation are supplied in selectively controlled amounts by gravity to negatively charged wells 34 by hose 50 from a tank 48 located on surface 14. Sufficient water is added to the wells to assure ability of bacteria to migrate in the earth material if the contaminants are not below the water table level. Water containing a few ounces of aerobic bacteria and nutrients including nitrates or urea and phosphates is fed by gravity into the bore hole through the hollow negative electrode, which is perforated with apertures 44. The electrodes are energized for several hours to stimulate or stress the microbes and to deliver the microbes and enzymes and nutrients

horizontally throughout the contaminated area to act upon the nutrients to perform an enzymatically catalyzed hydrolysis of the substrate in the presence of the water to provide availability of sufficient oxygen for a continuing aerobic bioremediation action on the contaminants even after the power from the welder is interrupted.

Evidence that migration of the nutrients and microbes take place was demonstrated across a 50 foot separation of electrodes after four hours of energization from the DC generator by a drop of nitrates from 145 ppm. to about 5 to 8 ppm. at the negative electrode while the nitrates at the positive electrode rose from less than 1 ppm. to about 15 ppm. Reduction of BTEX contaminant compounds was observed. Additional nutrients in a water solution can be periodically supplied to the negative well or both wells to maintain the aerobic bioremediation action on the contaminants. As in other embodiments of the invention, the enzymatically catalyzed hydrolysis of the substrate in the presence of water and the microbial action on the nutrients can continue under aerobic conditions without having to aerate or otherwise supply oxygen to the contaminated mass. These hydrolysis reactions in the presence of water continue long after the electrical energization of the water from the electrodes has been discontinued.

In another test across an electrode spacing of about 430 feet, 80 gallons of nutrient rich solution were fed to the negative electrode to a nutrient deficient contaminated mass of earth at a depth of 7 to 12 feet, the negative electrode being shielded for the first 7 feet and exposed to the contaminated 5 foot layer by means of perforations in the PVC pipe.

Between two vertical electrodes of opposite polarity,

the DC electric field as seen from above would be oval or like a football in shape. When using multiple electrodes these shapes can be made to overlap in any desired field pattern. One or more negative electrodes can be located at the center of a circular area, as in FIG. 7a, or along one side of a grid or network pattern, as in FIG. 7b, to define a horizontally extending area having one or more DC electric field patterns.

Satisfactory tests were performed using a Lincoln 200 DC welder having a 70 HP. engine and a DC rating of 200 amp. at 40 volts as a power source. During tests the welder was maintained at a setting of 110 amps., but the net current while running the tests was of the order of 3 to 5 amps.

Ex-Situ Bioremediation of Liquid Wastes

In addition to the in-situ bioremediation process just described where the remaining degraded constituents may typically be left in place, it is also possible to utilize techniques of this invention to perform ex-situ processing, i.e., moving a quantity of liquid waste or contaminated solid material or soil into a container for bioremediation.

The bioremediating product is believed to be suitable for bioremediation of categories of contaminants as follows:

I. Crude oils
   - A. Asphalt
   - B. Paraffin
   - C. Tar sands
   - D. Creosote

II. Chlorinated compounds
   - A. Trichloroethylene (TCE)
   - B. Perchloroethylene (PCE)

C. Methylene chloride

D. All other chlorinated solvents

E. Pesticides

1. DDT
2. DDD
3. DDE
4. Toxaphene
5. Dieldrin
6. Chlordane
7. Dichloroethane

F. Phenolics

G. PCB's

H. Herbicides

III. Glycols

A. Ethylene

B. Diethylene

C. Trimethylene

IV. Amines

A. Ethanolamine

B. Alkalinoamine

V. Paints

A. Enamel

B. Epoxy

C. Urethane

D. Xylene

E. Imron

VI. Automotive fluids

A. Transmission fluids

B. Brake fluids

C. Motor oils

VII. Tank bottoms

A. Crude oil tanks

B. Diesel oil tanks

VIII. Refinery sludges

A. Styrene

B. Paraffin

C. Urethane

D. Asphalt

E. Anthracene

F. Pyrine

G. Coal tars

Most, if not all, of these substrates can be treated in containers of different sizes dependent on the amount of material to be remediated.

EXAMPLE 2

A process for such treatment in accordance with one embodiment of the invention may be performed in an inert plastic 55 gallon drum of PVC material (see FIG. 10) for action on liquid substrates such as hydrocarbons. The drum is placed on end and filled to within about 10-12 inches of the open top with sand. About 5 to 15 gallons of the substrate liquid is mixed with the sand to coat the surface of the sand particles. Clear water is added to fill the barrel to a level a few inches above the sand/waste mix and a small quantity of aerobic seed microbes and a quantity of nutrients are added. Enzymatically catalyzed hydrolysis of the substrate in the presence of water and the microbial action on the nutrients takes place under aerobic conditions without having to aerate or otherwise supply oxygen to the mix. The color of the water in the top of the drum is a visual indicating means to verify

when the remediation process is complete. When the water color turns dark, indicating such completion, the liquid is removed from the drum except for about 6 inches in the bottom which serves as microbe seeding for additional processing. The remediated waste may be removed by means of a manually or remotely controlled drain valve at the periphery of the lower end of the drum. Separate drain valves can be used to facilitate on the one hand keeping a 6 inch level of substrate in the container for seeding, and on the other hand allowing complete removal of the remediated substrate. When the draining operation is completed, additional waste is added to the sand and preferably allowed to sit for about one hour to let the substrate migrate through the sand and coat the sand particles. Then the drum is again filled as above with clear water and nutrients again added for another processing operation.

Repeated bioremediation operations can be performed at regular intervals. This process lends itself to use in a manufacturing plant or the like where hazardous waste is being generated continuously as part of a continuing manufacturing or industrial operation. When the substrate to be bioremediated is a particular material, it is likely to be the case that one or more particular amino acid or fatty acid byproducts will be produced by enzymatic action on the substrate. The products drained from the drum after a processing operation may subsequently be passed through a separating process to isolate any desired amino acid or fatty acid product. Such products usually have a significant market value and their sale may constitute a significant recycling step and help reduce the cost of disposal of hazardous waste.

Among the types of bioremediation by-products produced when using the present invention are fatty acids which

are produced as a result of microbial action on the hydrocarbons and amino acids which are probably produced mostly as a result of the breakdown or death (lysis) of the microbes. It is apparent that there may be some real commercial potential in recovering fatty acids as remediation co-products. They are much more concentrated; they are present at a percent or so of the sample weight.

In the bioremediation of contaminated solids or soils the contaminated material may be added to the reactor without or mixed with sand or other solid media. The bioremediated solid material must then be periodically removed from the reactor. An example of the bioremediation of contaminated soil is discussed below.

Thus the costs of recovering them should not be excessive (production costs are assumed to be virtually nil). Second, the markets for fatty acids are very large and the most valuable fatty acids are the longer chain acids (C10 and greater), which by far dominate the mixture produced at the test site. In fact, it is likely that the mix of fatty acid products can be tailored somewhat by adjusting remediation conditions.

In-situ and ex-situ bioremediation of oil-contaminated soils by microorganisms in accordance with this invention produce mixtures of amino acids and fatty acids, both of which might potentially be recovered as valuable products. It is a well-known fact that microbial action on long chain hydrocarbons can produce organic acids of various chain lengths. In addition, as cells age, they eventually lyse, releasing their contents to the surroundings. Typically other microorganisms will then take up and use these released compounds. Therefore we can expect to find both organic acids and amino acids, the breakdown products of

proteins in areas where microbial populations are active on long chain hydrocarbons.

There are large, established markets for organic acids and amino acids. If these compounds can be economically recovered from bioremediation mixtures and sold into these markets, it may be possible to reduce or even eliminate the cost of site remediation.

In-Situ Bioremediation of Soils Employing an Open Pit

The process of this invention has been applied to a pit (see FIG. 9) measuring about 12 feet by 18 feet by 2 feet deep with a beginning total petroleum hydrocarbon level in the soil forming the pit of about 20 percent. A centrally located vetical tube is installed in the pit and extends into the earth below the pit to extract bioremediation products. The treatment was begun by forming a pond by adding about 40 barrels of water to the pit plus fertilizer plus about 10 ounces of the compost end product of this invention. Every few days, a dark-colored water was removed from the pit and then more clear water and fertilizer or nutrient were added.

Samples were taken to represent the undisturbed material prior to the test, the microbially-treated sludge at the bottom of the treated pond, the soil several feet below the bottom of the pond and a very deep sample from about 50 feet below the bottom of the pond. No liquid samples were taken.

The solid samples were analyzed by extraction with various solvents followed by analysis of the extract by different means to determine individual amino acids, total fat, and individual fatty acids (saturated and unsaturated). In most cases, the analytical results were reported as a percent of the unextracted sample. See Tables 5 and 6, below:

ANALYSIS	RESULTS
Total Fat	7.41 %
Protease	*N/D (None Detected)
Protein	56 mg/100 gm
Lipase	*N/D (None Detected)
Amino Acid Profile	
Aspartic Acid	1.63 mg/100 gm
Tyrosine	5.20 mg/100 gm
Valine	4.42 mg/100 gm
Methionine	4.87 mg/100 gm
Cysteine	1.12 mg/100 gm
Isoleucine	3.16 mg/100 gm
Leucine	3.33 mg/100 gm
Phenylalanine	4.51 mg/100 gm
Lysine	5..01 mg/100 gm
Glutamic Acid	0.84 mg/100 gm
Seine	2.20 mg/100 gm
Glycine	2.33 mg/100 gm
Histidine	3.19 mg/100 gm
Arginine	3.64 mg/100 gm
Threonine	2.12 mg/100 gm
Alanine	1.92 mg/ 100 gm
Proline	7.28 mg/100 gm
Saturated Fatty Acid	1.48 %
Monounsaturated Fat	0.76 %
Polyunsaturated Fat	0.22 %

Table 5: Amino Acids Produced in a Single In-Situ Bioremediation of Paraffin Crude Oil in an Earthen Pit

COMPOSITION	FORMULA	FRACTION %
	Fatty Acid Profile	
Caprylate	C8:0	0.74
Caprate	C10:0	6.94
Laurate	C12:0	5.43
Myristate	C14:0	4.68
Palmitate	C16:0	1.73
Palmitoleate	C16:1	1.33
Stearate	C18:0	1.21
Oleate	C18:1	4.86
Linoleate	C18:2	2.17
Linolenate	C18:3	0.47
Arachidate	C20:1	0.37
Eicosenoate	C22:0	<0.1
Behenate	C22:1	2.88
Erucate	C24:0	2.85
Lignocerate		2.18
Total Saturated Fatty Acid		3.34
Total Monounsaturated Fatty Acid		1.15
Total Polyunsaturated Fatty Acid		0.34
Total Fat		12.70

Table 6: Fatty Acids Produced in a Single In-Situ Bioremediation of Paraffin Crude Oil in an Earthen Pit

The levels of total solid phase fatty acids are approximately one percent by weight. Fatty acids are both volatile and sparingly soluble, providing the opportunity of separating and purifying them by the relatively simple (and inexpensive) techniques of distillation and fractional crystallization.

Specific By-Product Obtained from Bioremediation of Specific Substrate in Soils

The compost-produced bioremediation product of this invention is believed to be non-specific as to the substrates which can be treated and bioremediated thereby. However, it is further apparent that any byproducts produced by such remediation process will be substrate specific, i.e. the byproduct will depend on the composition of the substrate being broken down.

An example of production of an amino acid from a hazardous waste is the production of tyrosine from biodegradation of toxaphene-contaminated soil using microbial products of the present invention. Toxaphene is one of the compounds highly resistant to biological attack because of its high chlorine content, nearly 70 percent chlorine. See FIG. 8 for an illustration of a tank useful for bioremediating toxaphene-contaminated soil. The process steps employed are similar to those used in the ex-situ bioremediation of wastes described above. Clear water is added to the tank to a desired level (about 3 inches) above the contaminated soil. A small quantity of aerobic seed microbes obtained from the composting process of this invention and a quantity of nutrients are added. Enzymatically catalyzed hydrolysis of the substrate in the presence of water and the microbial action on the contaminant takes place under aerobic conditions without having to aerate or otherwise supply oxygen to the soil. The color of the water in the top of the tank turns dark and the liquid containing the bioremediation product, tyrosine is decanted or otherwise removed to the level of the soil. Additional water is added and the process repeated until sufficient bioremediation is accomplished. Toxaphene was a widely used pesticide until banned by EPA in the early 1980's. Analytical

results of the analysis of the bioremediation product of Toxaphene is shown in Table 7, below:

ANALYSIS	RESULTS
	AMINO ACID PROFILE
Alanine	None Detected
Alpartic Acid	None Detected
Tyrosine	53,000 ppm
Valine	None Detected
Methionine	None Detected
Cysteine	None Detected
Isoleucine	None Detected
Leucine	None Detected
Phenylalanine	None Detected
Lysine	None Detected
Hydroxyproline	None Detected
Glutamic Acid	None Detected
Serine	None Detected
Glycine	None Detected
Histidine	None Detected
Arginine	None Detected
Threonine	None Detected
Proline	None Detected
Protein	5.38 %
Saturated Fatty Acid	<0.1
Monounsaturated Fat	<0.1
Polyunsaturated Fat	<0.1
Total fat	0.30 %

Table 7: Analysis of Bioremediation Product of Toxaphene for Amino Acids

These results establish the specificity of the bioremediation reaction of the present invention when treating toxaphene, producing the amino acid tyrosine as the exclusive amino acid product.

The above procedure is useful for bioremediation of any contaminated soils or other solids conatminated with substrates amenable to bioremediation.

Theory of Operation of Bioremediation Process

A hydrolysis reaction may be catalyzed abiotically or biotically. Several sources confirm that hydrolysis proceeds faster when mediated by microbes. Enzymatic hydrolysis is expedited when enzymes hold reacting species in the "right positions" enhancing rates of interaction. By doing this, unfavorable changes in energies of activation are avoided. These compound-enzyme complexes are subsequently separated by attack with water. The end result is as if a direct attack on the complexes was performed exclusively by water. During operations using the unique compost products of the present invention for bioremediation of soil and ground water contaminated with petroleum and chlorinated compounds, data were collected as presented above showing that enzymatically catalyzed hydrolysis is a major mechanism of degradation during the treatment process in accordance with the present invention.

Moisture content was a limiting factor to both the rates (kinetics) and extent (thermodynamics) of reactions, as is expected during a hydrolysis reaction. The treatments showed apparent low substrate specificity. Dechlorination was usually accompanied by increased solubility also expected during a hydrolysis reaction. For example, theoretical toxaphene

solubilities increased 15 times (from 3 ppm to 45 ppm) during treatability studies. Nucleophilic dehalogenation by nucleophilic substitution of halogen groups is common during enzymatically catalyzed reactions, and results in increased solubilities due to hydroxylation.

The reactions mediated by the bacterial cultures of the present invention proceed faster than is currently expected using even advanced biotechnologies. Long chained aliphatic compounds are degraded almost as readily as short chained aliphatic compounds. High rates of reaction, in addition to dechlorination, solubilization, and degradation of various compounds (including "heavy" compounds like paraffin and asphalt) indicate that the reactions are facilitated by lowered energies of activation, an enzymatic phenomenon.

By-products of the bioremediation treatment according to the present invention include market-demand amino acids. Saturation of the treatment solutions with amines, amino and carboxylic acids, typical products of hydrolysis reactions, inhibited compound degradation. It appears that saturation of the treatment solution with polar byproducts neutralizes the active sites of the water molecule effectively reducing its reactivity. This is analogous to the low moisture content scenario. Compound degradation is resumed when the acids are extracted and replenished with fresh water for continued degradation. Salt water is believed to be even more effective.

Enzymatic hydrolysis is believed to be a major degradation mechanism used in the present process for treatment of contaminated soils. The enzymatic nature of the culture increases the rates of degradation and lowers substrate specificity of treatment. Because of the elevated levels of energy involved

during enzymatically catalyzed hydrolysis, technologies such as air sparging (blowing air in) and soil vapor extraction (replacing with fresh air) may well become obsolete.

Although the metabolic and enzymatic actions of the processes described in connection with the present invention take place for the most part in an anaerobic environment, the well known fermenting ammonia and undesirable odors which often accompany anaerobic processes are minimized. This is particularly desirable in the case of composting in land fills near populated municipal locations. It also is desirable to similarly minimize malodorous conditions at manufacturing or industrial sites.

Other variations within the scope of this invention will be apparent from the described embodiments and it is intended that the present description be illustrative of the inventive features encompassed by the appended claims.

CLAIMS:

1. An autothermal, thermophilic digestion process for degradation of a mass of typical municipal solid waste, said process characterized by the steps of:

A. Mixing said waste mass with manure and secondary sewage sludge to form a compost biomass pile, said manure and sewage sludge having sufficient moisture and indigenous bacteria, including aerobic bacteria, to carry out composting of said pile;

B. Allowing said compost pile to autothermally react for an initial time period, said initial time period being terminated upon said compost pile reaching a temperature of from about 115 degrees to about 140 degrees F.;

C. Adding to and distributing an alkaline material throughout said compost pile to quickly raise the pH of said pile to at least 10:

D. Allowing said compost pile to autothermally react for a second period of time, said second period being terminated upon said compost pile reaching a temperature of at least 180 degrees F.

E. Adding nutrients selected from the group comprising inorganic compounds of nitrogen, phosphorus, sulfur and potassium to said compost pile to support continuing metabolic reactions within said compost pile; and

F. Allowing said compost pile to further autothermally react for a third period of time, while adding water sufficient to maintain a moisture level in said pile of about 60 to about 82 percent throughout said third time period, said third time period terminating upon said biomass degrading to produce a non-pathogenic compost product;

G. Said reaction being carried out during said third period of time in the absence of mechanical aeration of the compost pile or the addition of gaseous oxygen into said compost pile.

2. The autothermal thermophilic digestion process according to claim 1, characterized in that said autothermal reaction within said third time period is an enzymatically catalyzed hydrolysis reaction.

3. The autothermal thermophilic digestion process according to claim 2, characterized in that said indigenous bacteria are stressed during said second time period such that a population of viable stressed aerobic bacteria survive to react during said third time period, wherein said first time period can be several hours, said second time period can be about 48 hours, and said third time period can be about six weeks.

4. The autothermic thermophilic digestion process of claim 1, characterized in that said nutrients comprise equal volumes of (21-0-0)ammonium sulfate, (0-45-0)super triple phosphate, and (0-0-60)potassium.

5. The composted fertilizer product of the process of claim 4, characterized in that the gypsum content is in the range of about 3 to about 12 percent by weight.

6. The composted fertilizer product of the process of claim 4, characterized by phosphogypsum in an amount corresponding to the amount of super triple phosphate added in said nutrient addition step.

7. The compost product of the process of claim 1 or of the process of claim 3.

8. A viable culture of aerobic bacteria produced according to the process of claim 1, which may be capable of

bioremediating chlorinated hydrocarbons and long chain hydrocarbons, including paraffin and asphalt, in the presence of nutrients and water to bioremediate said hydrocarbons by enzymatically catalyzed hydrolysis of the hydrocarbons.

9. An in-situ process for bioremediating a media containing hydrocarbon contaminants, said process comprising:

A. exposing the hydrocarbon contaminants in said media in-situ to a viable culture of aerobic bacteria according to claim 8;

B. Assuring the presence of sufficient nutrients and moisture to support bioremediation of said hydrocarbons;

C. Carrying out an enzymatically catalyzed hydrolysis of said hydrocarbon contaminants;

D. Periodically extracting a water solution containing bioremediation products from the situs of the in-situ bioremediation process; and

E. Adding fresh water to said situs in an amount approximately equal to the amount extracted in step D, above;

F. Said water extracting step D and said water addition step E being repeated in alternating steps until said site is sufficiently bioremediated.

10. A process according to claim 9, characterized in that said media is a subsurface earth material or said media is located in a pit.

11. An ex-situ process for bioremediating a material contaminated with organic or hydrocarbon contaminant materials within a container having a granular media therein, said process comprising:

A. Mixing the contaminated material with said

granular media;

B. Submerging the mixture in the container in water;

C. Adding a viable culture of aerobic bacteria according to claim 6;

D. Adding nutrients in a sufficient amount to support a bioremediating, enzymatically catalyzed hydrolysis reaction;

E. Extracting a water solution containing bioremediation products from said container; and

F. Adding fresh water to said container in an amount approximately equal to the amount extracted in step E, above;

G. Said water extraction step E, and said water addition step F being repeated in alternating steps until the contaminated material is sufficiently bioremediated.

12. A process according to claim 15, characterized in that said media is sand, and/or said contaminant is chlorinated hydrocarbons and long chain hydrocarbons, said long chain hydrocarbons including paraffin and asphalt.

13. A commercially useful amino acid product resulting from the bioremediation process of claim 12, and/or a commercially useful fatty acid product resulting from the bioremediation process of claim 12.

14. A system for in-situ subterranean bioremediation of a horizontally extending stratum in an earthen area (12) contaminated with organic or hydrocarbon material, said system characterized by:

A. At least two small diameter (18, 34) wells extending vertically from the surface (14) of said earthen area (12) to points at least immediately below said stratum

(16) of earth to be bioremediated;

B. Each said well (18, 34) having an outer tube (20, 36) enclosing a vertically extending electrode (26, 42) extending to the level of said stratum (16);

C. A direct current power source (30);

D. Means (4, 6) for connecting a first electrode (42) in a first one of said wells (34) to a negative terminal of said power source (30);

E. Means (32) for connecting a second electrode (26) in a second one of said wells (18) to a positive terminal of said power source (30);

F. At least the outer tube (36) of said one well (34) being perforated at the level of said stratum (16);

G. Means (48, 50) for supplying to said one well (34) sufficient aqueous mixture of bioremediating bacteria to allow a bioremediation reaction to take place;

H. Said bioremediation reaction being stimulated by said electrodes (26, 42), said electrodes being energized by said power source (30): and

I. Means to activate said power source (30) to provide an electric field between said electrodes (26, 42), whereby said water supported bioremediation reaction is caused to migrate from said first well (34) to said second well (18) within said stratum (16) to progressively bioremediate said stratum in-situ.

15. A system for in-situ subterranean bioremediation in accordance with claim 14, characterized in that at least said first electrode (42) in said first well (34) is an electrically conducting tube which has perforations (44) at the level of said stratum (16) and wherein at least part of said aqueous mixture is supplied

through said conducting tube.

16. A system for in-situ subterranean bioremediation in accordance with claim 15, characterized by multiple pairs of oppositely charged electrodes (26, 42) located in multiple respective wells (18, 34) for driving such aqueous mixtures across differing respective contaminated stratum portions.

17. A system for in-situ subterranean bioremediation in accordance with claim 14, characterized by a tank (48) at the surface of said site for controlled gravity feed therefrom of a portion of said mixture to said first well (34).

18. A method for in-situ subterranean bioremediation of a horizontally extending stratum (16) in an earthen area (12) contaminated with organic or hydrocarbon material, said method characterized by the steps of:

A. Introducing a mixture of water and bioremediating bacteria to a first location within said stratum (16);

B. Subjecting said stratum (16) to a direct current electric field having a positive pole and a negative pole, said positive pole being in the vicinity of said first location within said stratum; and

C. Allowing a bioremediation reaction to proceed at a reaction site in the vicinity of said first location, said reaction site being caused to migrate across said stratum (16) from said first location to a second location in the vicinity of said negative pole under the influence of said electric field.

19. The method for in-situ subterranean bioremediation of claim 18, characterized in that said bioremediating bacteria comprise a viable culture of aerobic bacteria according to claim 8 and said bioremediation reaction

occurs principally by enzymatically catalyzed hydrolysis of the organic or hydrocarbon contaminant material.

20. The method for in-situ subterranean bioremediation of claim 19, characterized in that water is added at said first location over the period of said bioremediation reaction.

21. A process for bioremediating an aqueous media containing paraffin crude oil contaminants comprising:

A. Preparing a first mixture of secondary digester sewage sludge having about 3 to about 5 percent moisture by weight and horse manure, said sludge acting as a wetting agent to form a pile;

B. Preparing a second mixture by adding paper product refuse to said aqueous media in an amount sufficient to absorb said media, thus forming an oily trash mix;

C. Mixing the mixture of step A with the mixture of step B to form a third mixture of "jello" like consistency having a moisture content of from about 60 to about 70 percent;

D. Allowing said third mixture to autothermally react substantially by means of enzymatically catalyzed microbial action until the temperature thereof reaches about 160 degrees F.

E. Turning said mixture from step D after about 10 days to expose interior portions of said pile to the exterior:

F. Allowing said mixture of step E to continue to autothermally react for about 20 days;

G. Turning said reacted mixture from step F to expose interior portions thereof to the exterior; and

H. Allowing said mixture from step G to

autothermally react for from about 30 to about 60 days until a compost product is attained.

22. The process of claim 21, characterized in that said aqueous media is located in an earthen pit and said second mixture is formed in-situ by adding said paper refuse directly to said pit.

FIG. 1

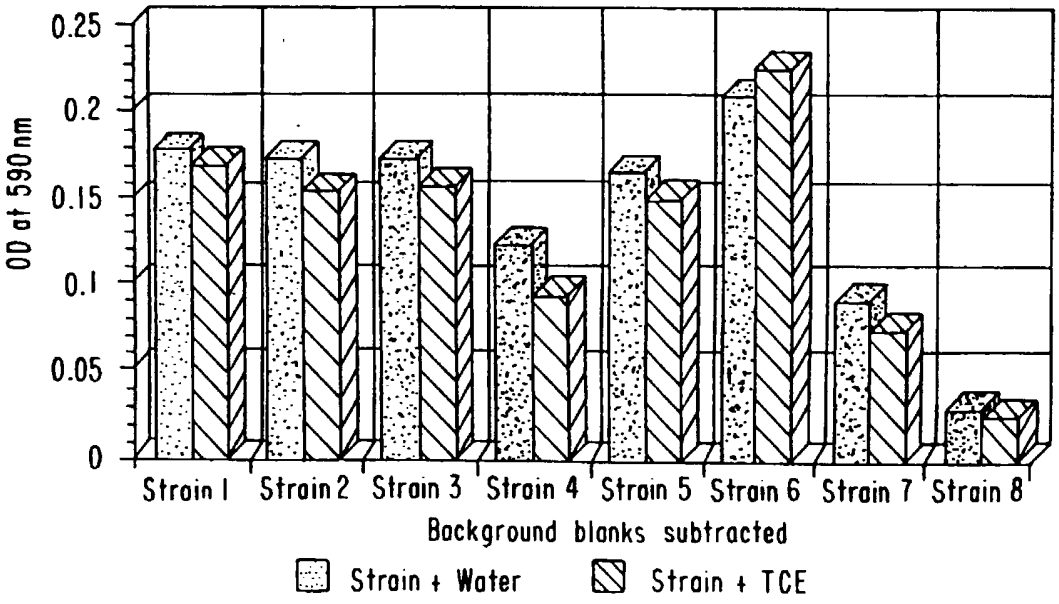


FIG. 2

48 Hour anaerobic endpoint assay on 8 strains using TCE

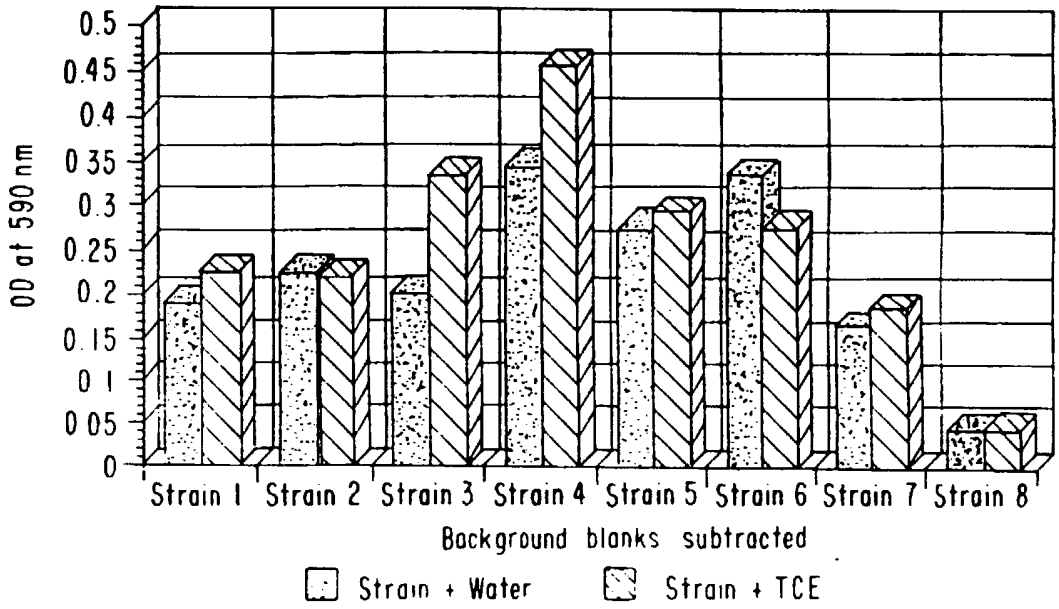


FIG. 3

48 Hour aerobic endpoint assay on 8 strains using TCE

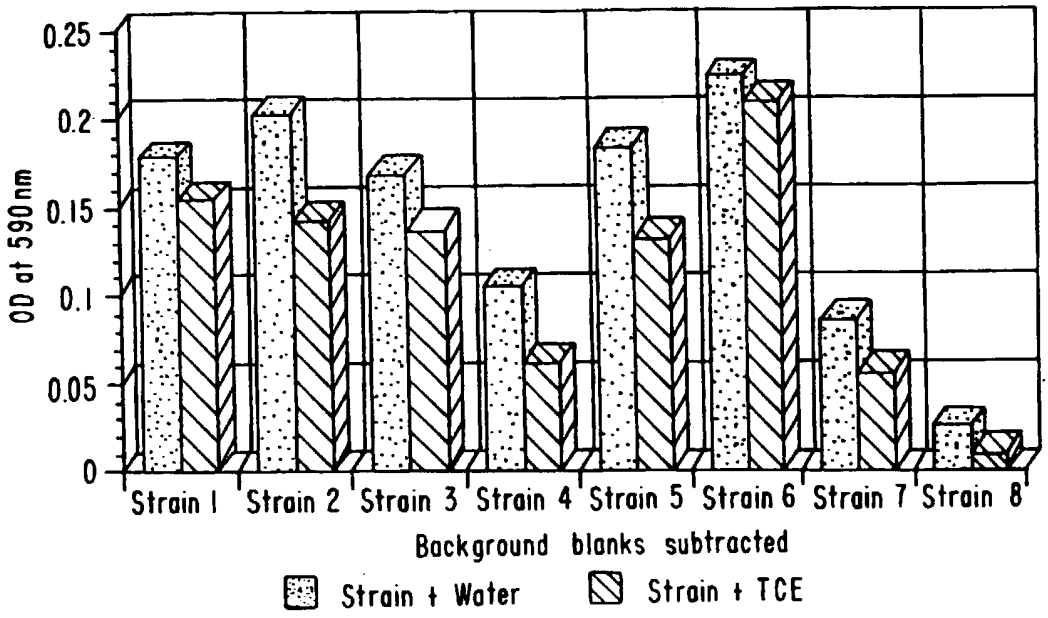
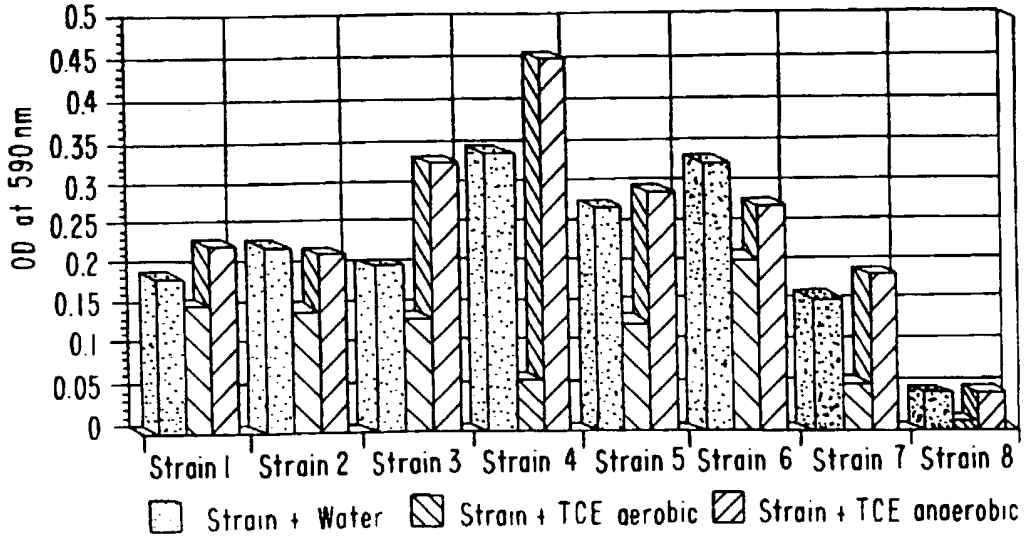


FIG. 4

48 Hour endpoint assay results showing both aerobic and anaerobic data



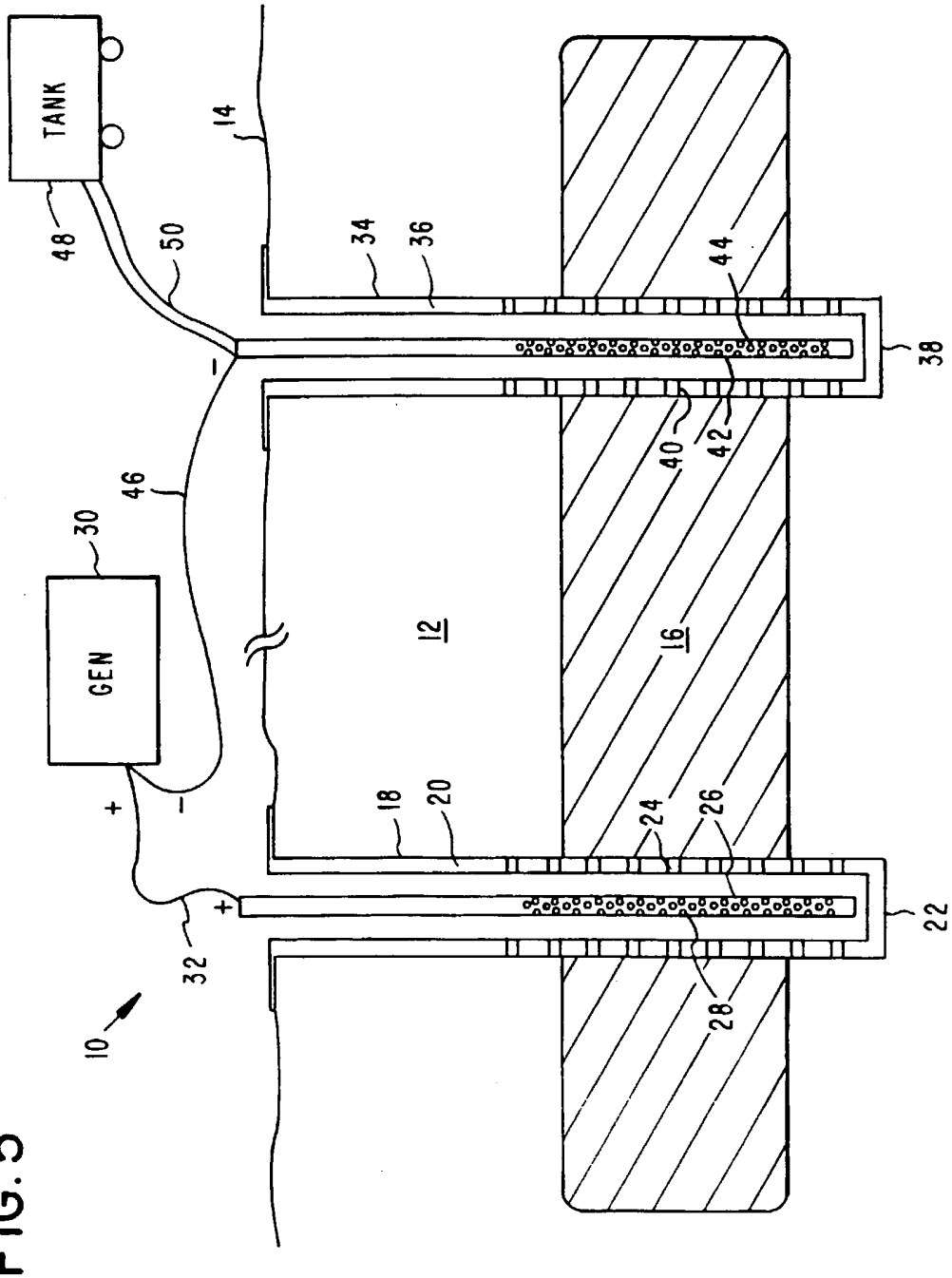


FIG. 5

FIG. 6

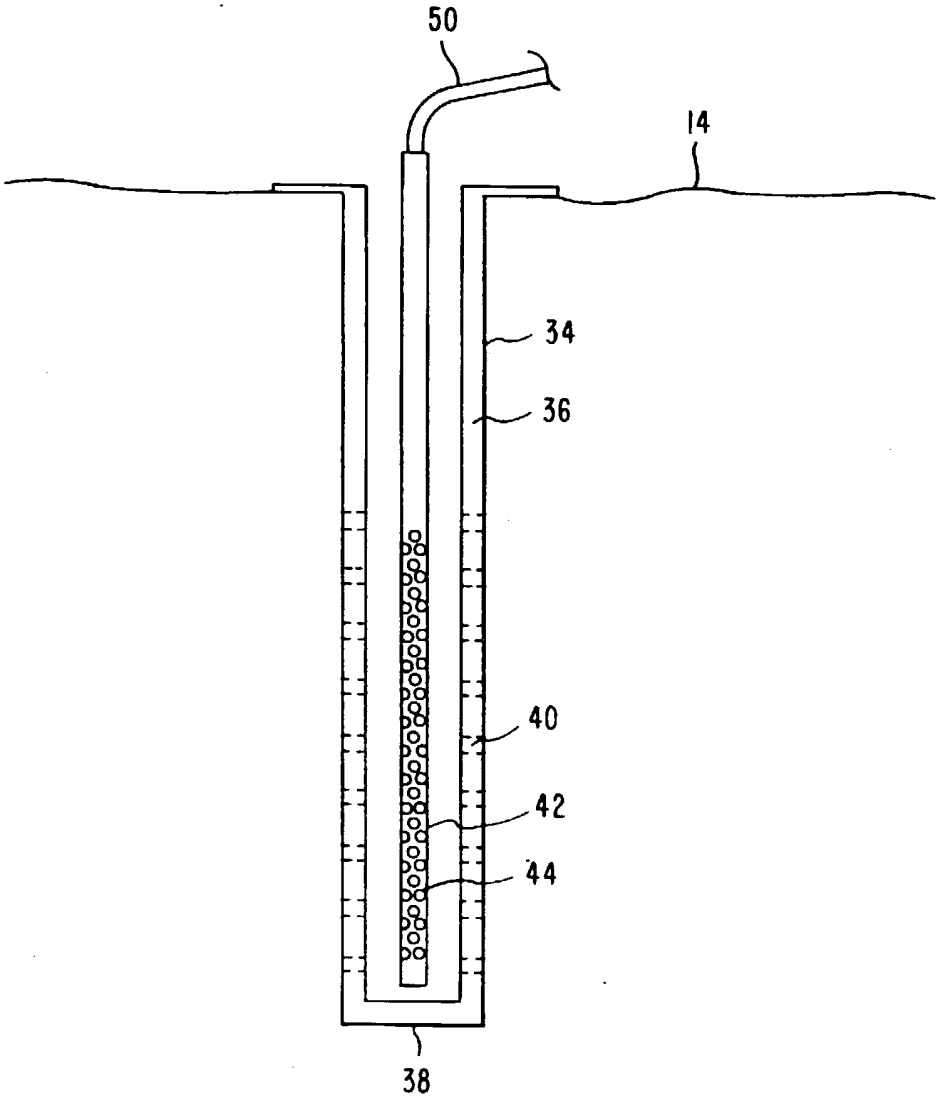


FIG. 7A

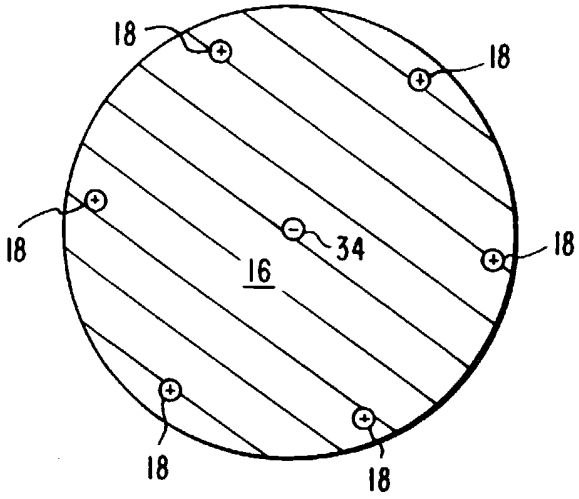


FIG. 7B

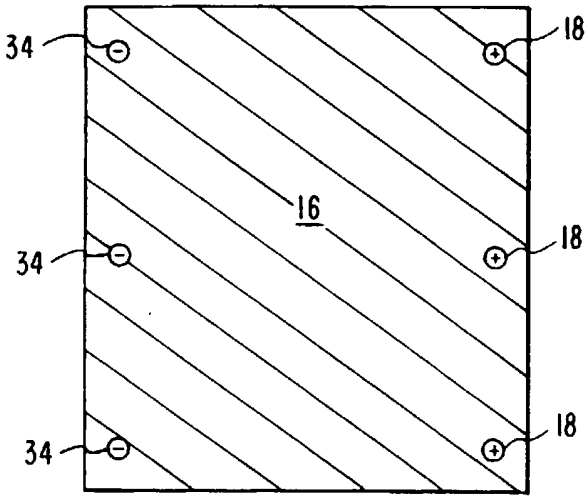


FIG. 8

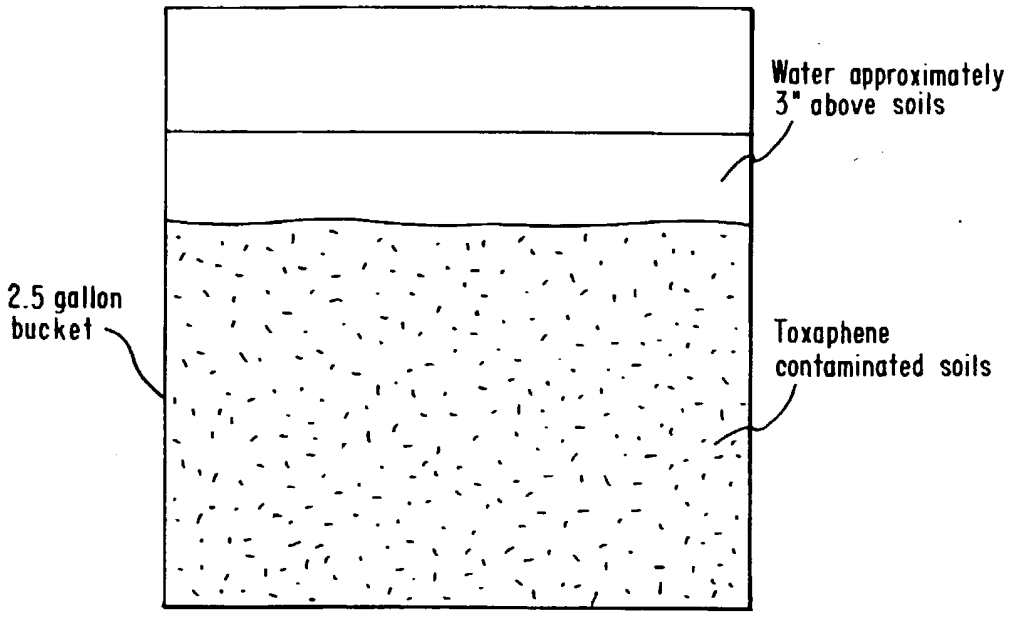

Water approximately
3" above soils
2.5 gallon
bucket
Toxaphene
contaminated soils

FIG. 9

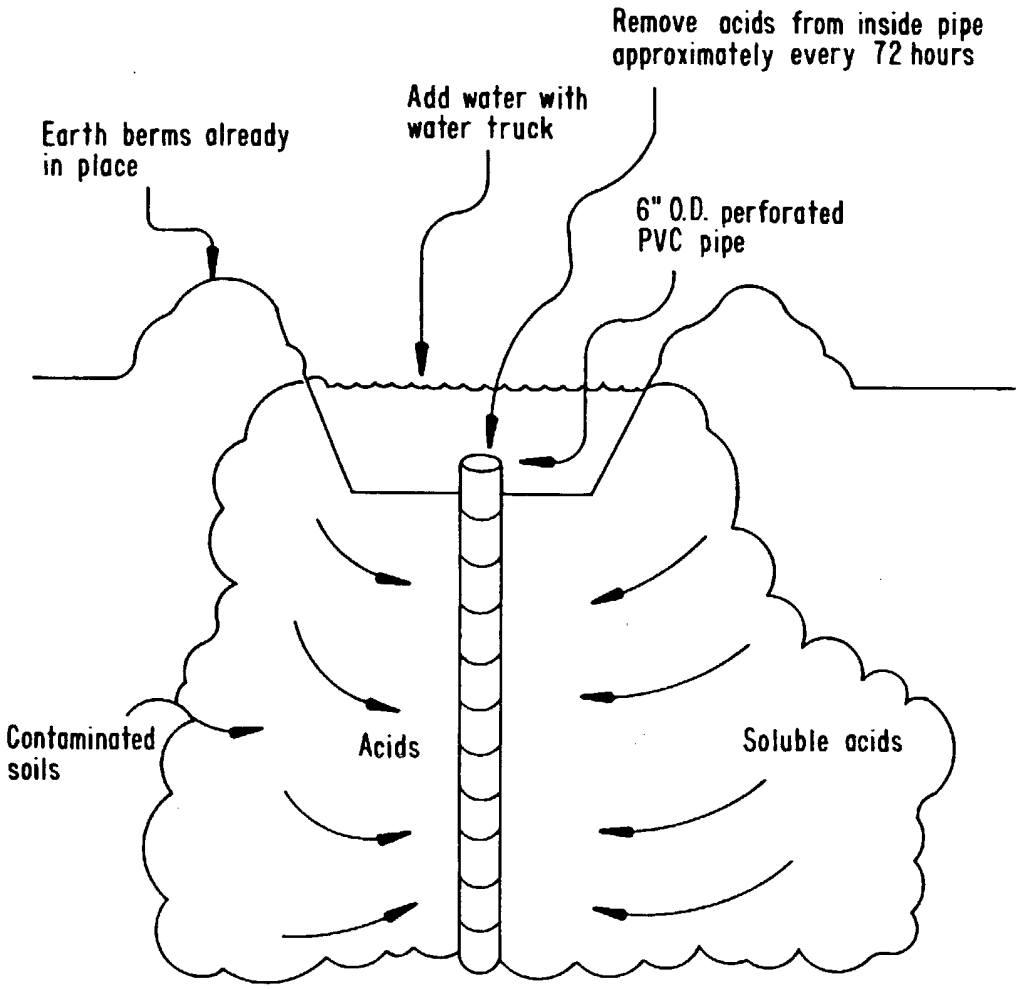
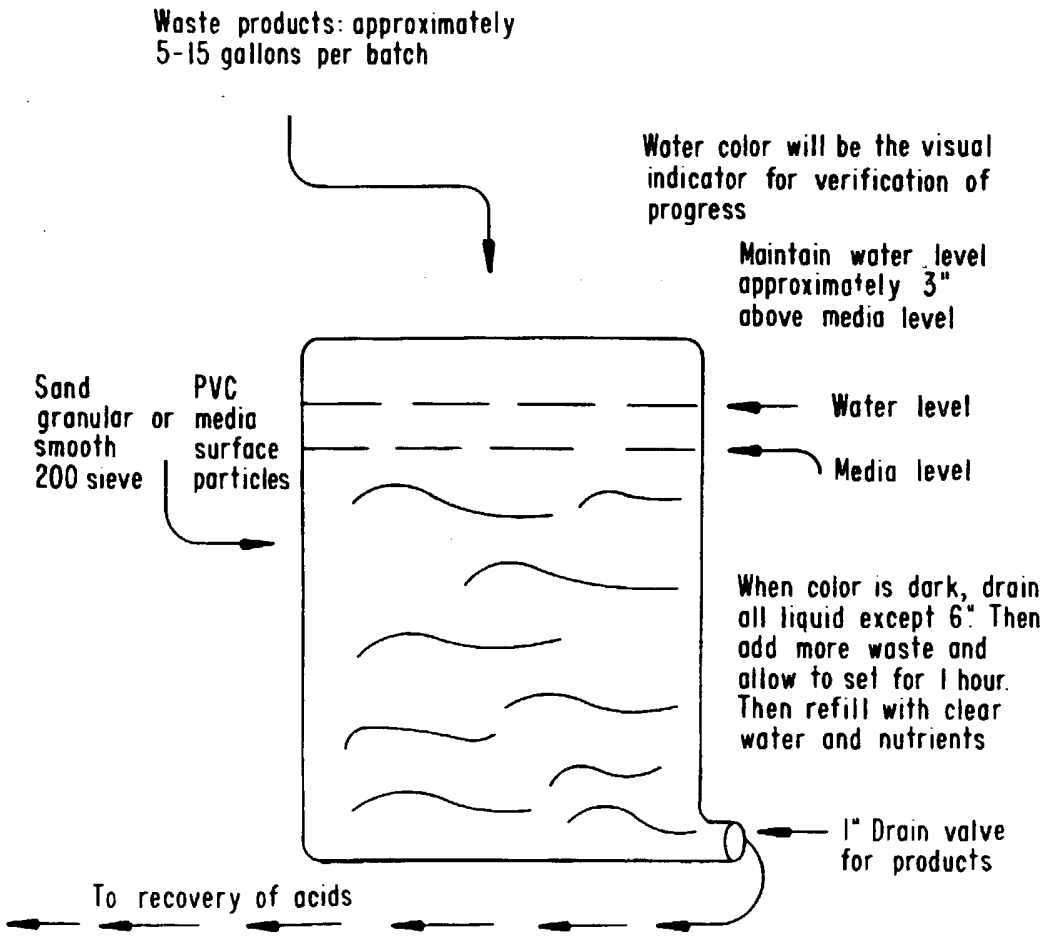

Remove acids from inside pipe approximately every 72 hours
Add water with water truck
Earth berms already in place
6" O.D. perforated PVC pipe
Contaminated soils
Acids
Soluble acids

FIG. 10


Waste products: approximately 5-15 gallons per batch
Water color will be the visual indicator for verification of progress
Maintain water level approximately 3" above media level
Sand granular or smooth 200 sieve
PVC media surface particles
Water level
Media level
When color is dark, drain all liquid except 6". Then add more waste and allow to set for 1 hour. Then refill with clear water and nutrients
1" Drain valve for products
To recovery of acids

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03775

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C05F 1/00, 3/00, 9/00, 11/00
US CL :71/11, 12, 13, 14, 15, 23, 24, 25, 901
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 71/11, 12, 13, 14, 15, 23, 24, 25, 901

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,743,287 A (E. C. ROBINSON) 10 May 1988, see entire document.	1-7
Y	US 5,169,782 A (J. MURPHY ET AL.) 08 December 1992, see entire document.	1-7
Y, P	US 5,466,273 A (L. V. CONNEL) 14 November 1995, see entire document.	1-7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 JUNE 1996	Date of mailing of the international search report 02 AUG 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RALPH GITOMER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03775

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03775

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s)1-7, drawn to a digestion process and product.
Group II, claim(s) 8, drawn to a bacterial culture.
Group III, claim(s) 9-13, drawn to bioremediating process and product.
Group IV, claims 14-20, drawn to in situ subterranean bioremediating.
Group V, claims 21-22, drawn to aqueous bioremediating.
The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The product and process of Group I is independent and does not require any feature of Group II. The bioremediating processes of Groups III, IV and V are each distinct methods within a single general inventive concept and independent of Groups I and II where they are not linked by a special technical feature within the meaning of PCT Rule 13.
PCT Rules 13.1 and 13.2 do not provide for multiple distinct methods and products with a single general inventive concept.